PATENT
Attorney Docket No.: A-57496-1/DJB

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re application of: | ) | Examiner: ZISKA, S. |
| WEISS, et al. | ) | Group Art Unit: 1804 |
| Serial No. 08/385,404 | ) | |
| Filed: February 7, 1995 | ) | |
| For: REMYELINATION USING NEURAL STEM CELLS | ) | |

DECLARATION UNDER 37 CFR 1.132

Commissioner of Patents
and Trademarks
Washington, DC 20231

Sir:

The undersigned, Brent A. Reynolds, hereby declares and states that:

1. I am a co-inventor of the subject application and I have read the arguments in the Official Action (mailed September 7, 1994) to U.S. Ser. No. 07/961,813, of which the captioned-application is a continuation.

2. The Examiner has rejected claims 1-17 and 25-28 and 30 under 35 U.S.C. 102(b) as being anticipated by Hunter *et al.*. It is the Examiner's opinion that because the cell culture method of Hunter *et al.* starts with brain cells of neonatal rats, it "inherently discloses stem cells capable of differentiating into neurons, astrocytes and oligodendrocytes." However the method of claims 1, 7, and 25 (and the claims dependent thereon) require that neural stem cells proliferate in culture. While the starting material of Hunter *et al.* may have contained neural stem cells, there is no indication from the article that neural stem cells were induced to proliferate using their methods. There is only a discussion about proliferation of O-2A progenitor cells.

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3. Under my supervision, experiments were performed in the laboratories at Neurospheres Ltd., Calgary, Canada, to test whether the culture conditions of Hunter *et al.* induce the proliferation of neural stem cells. Primary neurospheres were generated using the methods described in Example 1 of the specification. The neurospheres (known to contain multipotent neural stem cells) were harvested, dissociated to form a single cell suspension, and cultured at $6.3 \times 10^6$ cells per T25 flask (same density as Hunter and Bottenstein) using the following conditions:

1. Medium: Hormone medium (see p. 16, lines 18-26) + 20 ng/ml EGF
   Substrate: None
   Comment: Applicants' passaging culture conditions.

2. Medium: 67% O3 + 33% Conditioned Medium (CM)
   Substrate: Poly-D-Lysine, fibronectin
   Comment: Hunter's & Bottenstein's passaging culture conditions.

B104 cells, for preparation of the conditioned medium, were obtained from Dr. David Schubert at the Salk Institute [Schubert *et al.*, Nature 249:224-227 (1974)], and maintained as per Bottenstein *et al.* ["CNS neuronal cell line-derived factors regulate gliogenesis in neonatal rat brain cultures" J. of Neuroscience Res. 20:291-303 (1988)]. Culture of cells in 33% B104-conditioned N4 medium and 67% O3 medium was done per the methods of Hunter and Bottenstein ["Growth factor responses of enriched bipotential glial progenitors, Developmental Brain Res. 54:235-248 (1990)].

4. After 4 days in culture, many attached neurospheres (indicating neural stem cell proliferation), were observed in flasks cultured under condition 1. In flasks cultured under condition 2, no clusters of cells were observed. Instead, attached multipolar cells resembling oligodendrocytes and cells resembling astrocytes were observed on the floor of the flask. There

was no indication that the neural stem cells known to be present in these flasks had been induced to proliferate.

5. These results indicate that the use of conditioned medium from the B104 cell line does not cause neural stem cells to proliferate, despite the fact that this conditioned medium is reported to cause the expansion of O-2A progenitor cells obtained from the brains of neonatal rats. Although the conditioned medium is likely to contain some unidentified growth factors, the factors which are present may not be able to cause neural stem cell proliferation, or they may occur in levels which are insufficient to induce stem cell proliferation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that willful, false statements may jeopardize the validity/enforceability of the application or any patent issued thereon.

Dated: Sept 26/95 Signature: 

Brent A. Reynolds